

**In-vitro activity of chlorhexidine, hexetidine and bacitracin against perinatal pathogens**

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Sir,

To prevent microbial contamination and subsequent infection of the newborn during birth, it would be desirable to have a safe and efficacious antimicrobial agent that could be applied to the vaginal mucosa to prevent transmission of neonatal pathogens during labour. We studied the in-vitro activities of three possibly useful agents: chlorhexidine, hexetidine and bacitracin against clinical isolates of *Chlamydia trachomatis* (5 strains), *Ureaplasma urealyticum* (4), *Mycoplasma hominis* (4), *Streptococcus agalactiae* (50), *Escherichia coli* (50) and *Listeria monocytogenes* (10). Reference strains were *C. trachomatis* ATCC VR348B, *U. urealyticum* ATCC 27618 and ATCC 27813, *M. hominis* ATCC 14027 and ATCC 23114, *S. agalactiae* ATCC 13813 and NTCC 8181, *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213.

Susceptibility testing of *S. agalactiae*, *E. coli*, *L. monocytogenes* and *S. aureus* was performed by broth dilution in microtitre plates according to the guidelines of the NCCLS. Bacteria were grown on blood agar overnight at 37°C. Serial dilutions of the three test drugs were prepared in Iso-sensitest broth (Oxoid CM 473, Basingstoke, UK) in microtitre plates containing final concentrations of 0.25-1024 mg/L of chlorhexidine, 4-256 mg/L of hexetidine and 1.0-8192 mg/L of bacitracin per well; 100 µL of each dilution were mixed with an equal volume containing 10<sup>4</sup> cfu of each bacterial strain in broth. Plates were incubated for 18 h at 37°C and the MIC was read as the lowest concentration of each drug completely inhibiting growth.

Strains of *M. hominis* were grown in 65.5 mL PPLO broth (Difco 0554, Detroit, USA) supplemented with 10 mL yeast extract, 10 mL horse serum, 10 mL L-arginine hydrochloride, 1.5 mL thallium acetate, 0.002% w/v phenol red and 1000 IU/mL penicillin (final pH 7.0). Strains of *U. urealyticum* were cultured in 90 mL 3% w/v Trypticase Soy broth (Oxoid CM 129, Basingstoke, UK) supplemented with 3.6 mL yeast extract, 24 mL inactivated horse serum, 3 mL 40% w/v urea, 0.002% w/v phenol red, 40 mg/L cefuroxime and 4 mg/L amphotericin B (final pH 6.0). A suspension of 20,000 colour changing units/mL was mixed with 1 mL of each dilution of each drug, incubated for 30 min at 37°C and diluted 100-fold in broth medium. One mL was then

pipetted into four culture tubes which were incubated for 72 h at 37°C. The MIC was read as the lowest concentration of each drug to inhibit growth in all four tubes.

Strains of *C. trachomatis* were grown on McCoy cells in flat bottom tubes as described by Heessen & Muyltjens (1984). Growth medium consisted of Eagle's MEM with HEPES 20mM, 3% inactivated fetal bovine serum, 6g/L glucose, 50 mg/L gentamicin, 100 mg/L vancomycin, 2.5 mg/L fungizone and 1 mg/L cycloheximide. After inoculation, centrifugation (60 min at 3000 rpm) and incubation (72 h at 37°C), cultures were homogenized, fixed with methanol and stained with monoclonal antibodies for demonstration of inclusion bodies by immunofluorescence. A suspension of 20,000 inclusion forming units/mL was mixed with 1 mL of each dilution of test drug, left for 30 min, diluted 100-fold in chlamydia growth medium and 1 mL was inoculated on to each of four culture tubes and screened for inclusions after 72 h at 37°C. The MIC was defined as the lowest concentration of each drug completely inhibiting formation of inclusions.

The results of the in-vitro tests are given in the Table. Hexetidine did not inhibit *C. trachomatis* and *E. coli*, but was highly active against the other organisms; bacitracin only inhibited Gram-positive organisms; all strains were susceptible to 512 mg/L chlorhexidine or less. Although hexetidine was found to reduce the vaginal microflora by preoperative application (Wewalka *et al.*, 1991, Weidinger *et al.*, 1991), we think that it would be unsuitable for perinatal antiseptics, it not being active against *E. coli* and *C. trachomatis* which are significant neonatal pathogens. The same conclusion was made for bacitracin. For chlorhexidine we calculated that 10 mL of 1% chlorhexidine solution would result in local concentrations of 0.4 mg/cm<sup>2</sup> assuming the surface area of the normal vagina during labour being about 250 cm<sup>2</sup>. This concentration is far above the highest MIC for the organisms tested, being the volumetric MIC of 512 mg/L equivalent to 0.064 mg/cm<sup>2</sup>. This is in agreement with the experience that chlorhexidine can indeed prevent neonatal transmission of *S. agalactiae* (Kollée *et al.*, 1989; Burman *et al.*, 1992). Chlorhexidine has been used in obstetrics and gynaecology for several purposes and is not absorbed to any large extent (Vorherr *et al.*, 1984), making it a safe substance. It may be of interest to study the efficacy of routine vaginal application of chlorhexidine in the prevention of several neonatal infections.

**Table.** In-vitro activities of chlorhexidine and hexetidine against *C. trachomatis*, *U. urealyticum*, *M. hominis*, *S. agalactiae*, *E. coli*, and *L. monocytogenes* and of bacitracin against *S. agalactiae* and *L. monocytogenes*

Antimicrobial agent and organism (no. tested)	Cumulative number of organisms susceptible to concentrations (mg/L) indicated													
	0.25	0.5	1	2	4	8	16	32	64	128	256	512		
<b>Chlorhexidine</b>														
<i>C. trachomatis</i> (6)							1	4*	6					
<i>U. urealyticum</i> (6)												6*		
<i>M. hominis</i> (6)														
<i>S. agalactiae</i> (52)	1	43	51*		52*									
<i>E. coli</i> (51)			20*	50	51									
<i>L. monocytogenes</i> (10)			4	10										
<i>S. aureus</i> ATCC 29213			1											
<b>Hexetidine</b>														
<i>C. trachomatis</i> (6)	4	8	16	32	64	128	256							
<i>U. urealyticum</i> (6)							0							
<i>M. hominis</i> (6)						6*								
<i>S. agalactiae</i> (52)	12	52*												
<i>E. coli</i> (51)							0							
<i>L. monocytogenes</i> (10)		10												
<i>S. aureus</i> ATCC 29213		1												
<b>Bacitracin</b>														
<i>S. agalactiae</i> (52)	1	2	4	8	16	32	64	128	256					
<i>L. monocytogenes</i> (10)	3*	4	7	18	31	48	51	52*						
<i>S. aureus</i> ATCC 29213								5	10					
							1							

\*Indicates MIC of reference strain.

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### References

- Burman, L. G., Christensen, P., Christensen, K., Fryklund, B., Helgesson, A. M., Svenningsen, N. W. *et al.* (1992). Prevention of excess neonatal morbidity associated with group B streptococci by vaginal chlorhexidine disinfection during labour. *Lancet* **340**, 65-9.
- Heessen, F. W. A. & Muytjens, H. L. (1984). In vitro activities of ciprofloxacin, norfloxacin, piperidic acid, cinoxacin, and nalidixic acid against *Chlamydia trachomatis*. *Antimicrobial Agents and Chemotherapy* **25**, 123-4.
- Kollée, L. A. A., Speyer, I., van Kuijk, M. A. P., Koopman, R., Dony, J. M., Bakker, J. H. *et al.* (1989). Prevention of group B streptococci transmission during delivery by vaginal application of chlorhexidine gel. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **31**, 47-51.
- Vorherr, H., Vorherr, U. F., Mehta, P., Ulrich, J. A. & Messer, R. H. (1984). Antimicrobial effect of chlorhexidine and povidone-iodine on vaginal bacteria. *Journal of Infection* **8**, 195-9.
- Weidinger, H., Passloer, H. J., Kovacs, L. & Berle, B. (1991). Advantage of prophylactic vaginal antiseptics by hexetidine in obstetrics and gynecology. *Geburtshilfe und Frauenheilkunde* **51**, 929-35.
- Wewalka, G., Dorninger, G., Enzelsberger, H., Riel, T. & Rotter, M. (1991). Antimicrobial efficacy of procedures for preoperative vaginal antiseptics. *Hygiene und Medizin* **16**, 335-45.
- Paradoxical susceptibility to cephalothin and cefamandole of a *Klebsiella pneumoniae* isolate producing extended-spectrum  $\beta$ -lactamase (TEM-3).**
- J Antimicrob Chemother* 1996; **37**: 194-196
- Sir,  
Extended-spectrum  $\beta$ -lactamases (ESBL) are plasmid-mediated enzymes which confer resistance to most  $\beta$ -lactam antibiotics, including first-generation cephalosporins and oxymino- $\beta$ -lactams such as cefotaxime, ceftazidime and aztreonam. Since their discovery in the mid 1980s, about 40 enzymes have been identified, most of them derived by one or more amino acid substitutions from TEM or SHV enzymes. Most ESBL are inhibited by clavulanic acid, tazobactam or sulbactam, and can be readily detected by the double-disc synergy test showing that  $\beta$ -lactam activity is restored by the addition of clavulanic acid (Philippon, Arlet & Lagrange, 1994). *Klebsiella pneumoniae* is the most common bacterial species in which clavulanate-sensitive ESBL have been identified, and outbreaks of hospital-acquired infections involving multiresistant strains of this organism have been reported (Philippon *et al.*, 1994).
- During a survey of intestinal carriage of *K. pneumoniae* resistant to third-generation cephalosporins in patients hospitalized in a surgical intensive care unit (Hôpital Boucicaut, Paris), a strain (BOU 92) producing an unusual ESBL was isolated from a patient by cultivating stools on Drigalski agar containing 2 mg/L cefotaxime. Surprisingly, this isolate, although resistant to amoxicillin, ticarcillin, piperacillin, cefotaxime, ceftazidime and aztreonam, was susceptible to cephalothin and cefamandole (Table). In addition, strain BOU 92 was resistant to aminoglycosides (except for gentamicin), trimethoprim, sulphonamides, tetracycline, fosfomycin and quinolones. Resistance to  $\beta$ -lactams was transferred by conjugation from *K. pneumoniae* strain BOU 92 to *Escherichia coli* K-12 recipient strain HB101 and the recombinant strain was designated MS 92.1. Unlike the *Klebsiella* donor strain, the *E. coli* transconjugant was resistant to cephalothin and cefamandole (Table). Resistance to  $\beta$ -lactams was then transferred by conjugation from the *E. coli* transconjugant MS 92.1 to a wild-type strain of *K. pneumoniae* (JLG III.10) and the *K. pneumoniae* transconjugant (strain MS 92.2) exhibited the same phenotype as the *E. coli* donor strain (Table).
- BOU 92, MS 92.1 and MS 92.2 all produced an identical plasmid-mediated  $\beta$ -lactamase with pI of 6.3 as determined by isoelectric focusing on polyacrylamide gel of crude cell sonic extracts with TEM-1 and TEM-3 enzymes as pI markers (Jarlier *et al.*, 1988). The  $\beta$ -lactam resistance pattern and the enzyme pI strongly suggested that the ESBL produced was TEM-3; this assumption was confirmed by gene amplification with primers